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滇产对叶百部中一个新的百部生物碱*

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摘要：从对叶百部（*Stemona tuberosa*）根的乙醇提取物中分离鉴定1个新的百部生物碱，命名为去氢异滇百部碱（**1**），以及1个已知化合物：异滇百部碱（**2**）。它们的化学结构通过现代波谱解析得以鉴定。

关键词：对叶百部；百部生物碱；去氢异滇百部碱
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A New Alkaloid from the Roots of *Stemona tuberosa* (Stemonaceae)*

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Abstract: One new Stemona alkaloid, dehydroisostemotinine (**1**) along with one known alkaloid isostemotinine (**2**), were isolated from the roots of *Stemona tuberosa* collected from Yunnan Province. The structure of the new alkaloid was established on the basis of one- and two-dimensional NMR spectra and other spectroscopic studies.

Key words: *Stemona tuberosa*; Stemona alkaloids; Dehydroisostemotinine

Stemona represents the largest genus within the monocotyledon family Stemonaceae and is indigenous to southwest Asia and north Australia (Greger, 2006). Several species of this genus called Baibu have been used as Chinese medicine particularly for the treatment of respiratory diseases and against enteric helminthes or ectoparasites on human and cattle since ancient times (Jiangsu New Medical College, 1977).

Highly interesting secondary metabolites have been reported from *Stenoma* within the last 20 years. The Stemona alkaloids, the main constituents of the genus *Stemona*, were interesting not only due to its pharmacological profile but also due to complex and unusual molecular structures. More than 100 derivatives have been identified, all of them bearing a pyrrolo or pyrido [1, 2-α] azepine backbone unrealized in any other plant family (Sturm *et al.*, 2008). A number of studies have been performed to evaluate the biological activities of these unique alkaloids.

The species *S. tuberosa* (Stemonaceae), distributed widely in the southern China, is one of three authentic plant resources for Baibu in China pharmacopoeia (Chinese Pharmacopoeia Commission, 2005). Many investigations on *S. tuberosa* of different localities have led to the isolation of more than 60 alkaloids, which are classified into tuberostemonine-type, stemonine-type, and croomine-type. However, the major alkaloids of *S. tuberosa* vary greatly in samples collected from different locations, which

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causes problems when attempting to determine the relationship between the specific medicinal use and the herbal growing locations. Thus, it is necessary to further investigate for this species.

In our studies on the chemical constituents of the roots of *S. tuberosa* collected from Wenshan country, Yunnan Province, one new alkaloid, dehydroisostemotinine (**1**) and one known alkaloid isostemotinine (**2**) were isolated and identified. The present paper described their isolation and structure determination.

Results and Discussion

The structure of the new alkaloid1 **1** was established by mass-spectrometric and spectroscopic analyses, especially 2D-NMR techniques (^{1}H,^{1}H-COSY, HMBC, NOESY). Compound **2** was elucidated by comparing it spectroscopic data with the data reported in literature (Xu *et al.*, 1982).

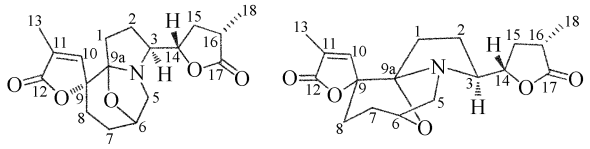

Fig. 1 Structure and configuration of compound **1**

Compound **1** was obtained as an optically active, colorless, amorphous power. Its molecular formula was established as $C_{18}H_{23}NO_5$ by HR-EI-MS (m/z 333.1579 ([M]$^+$; calc. 333.1576), inferring eight degrees of unsaturations. The ^{1}H NMR spectrum of **1** showed signals for one methane and two geminal protons attached to carbon atoms bearing a nitrogen functionality at δ 2.87 (1H, dd, J =6.8, 6.4 Hz, H-3), 3.28 (1H, dd, J =10.0, 15.0 Hz, H-5a), and 3.03 (1H, dd, J =15.1, 11.4 Hz, H-5b), which are characteristic of the pyrido [1, 2-α] azepine nucleus of the *Stemona* alkaloids (Jiang *et al.*, 2006). In the ^{1}H and ^{13}C NMR spectra, the signals of a secondary methyl at δ 1.26 (3H, d, J =7.5 Hz, H-18) indicated the presence of α-methyl-γ-lactone ring, an allylic methyl at δ 1.93 (3H, s, H-13) and an olefinic proton at δ 7.31 (1H, s), together with two olefinic carbon signals at δ 130.4 and 150.4, indicated the presence of an unsaturated α-methyl-γ-lactone instead of a typical α-methyl-γ-lactone moiety. This was confirmed by two pairs of lactone carbonyl and oxygenated carbon signals [δ 173.8 (C-12, s) and δ 87.9 (C-9, s); δ 179.4 (C-17, s) and δ 83.4 (C-14, s)]. All these data revealed that compound **1** shared the same basic skeleton of croomine. In succession, a detailed analysis of the NMR spectra revealed the planar structure of compound **1**.

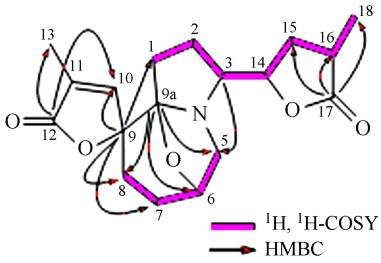

Fig. 2 HMBC and ^{1}H-^{1}H COSY correlations of compound **1**

On the basis of the HSQC and ^{1}H-^{1}H COSY spectra of **1**, there were three spin systems: from H-5 to H-8, H-1 to H-2 and H-14 to Me-18. The HMBC correlations of H-15, H-16 and Me-18 to C-17, H-3 and H-15 to C-14 indicated that an α-methyl-γ-lactone ring is located at C-3. In the HMBC, the correlations from the olefinic proton at δ 7.31 (1H, s, H-10) to C-8, C-9, C-9a, C-11, C-12, and C-13, suggested that C-9 is spiro-atom connecting the unsaturated α-methyl-γ-lactone and the pyrido [1, 2-α] azepine nucleus. The tetracyclic skeleton, plus the two lactonic carbonyls and one olefinic group, account for seven of eight units of unsaturation revealed by the molecular formula. Based on these data, compound **1** should have one more ring. The HMBC correlations between the proton at δ 4.68 (H-6) and δ 106.6 (C-9a) indicated that both C-6 and C-9a were linked by an O-atom to form a pyrido [1, 2-α] azepine ring. This structural moiety, the ether functionality between C-6 and C-9a has been observed before only in stemotinine and isostemotinine. Thus, the planar structure of **1** was figured out.

The relative configuration of **1** was established by the comparison the ^{1}H and ^{13}C NMR

data with compound **2**, and also confirmed by its ROESY experiment. The similar chemical shifts and in combination with biogenetic considerations suggested that these two compounds possess the same configurations. Accordingly, the structure of **1** was established as dehydroisostemotinine that the perhydroazaazulene ring has an "8-CH_2-down conformation" (Xu *et al.*, 1982).

Experimental

General Experimental Procedures MPLC: Büchi Pump Module C-605, and Büchi Pump Manager C-615. Column chromatography (CC): Silica gel H (10－40 mm; Qingdao Marine Chemical Ltd. Co.), amino silica gel (90－140 mm, FujiSilysia Chemical Ltd.) and Sephadex LH-20 (40－70 mm, Pharmacia). TLC: On silica-gel plates; spots were visualized by spraying with *Dragenoff's* reagent. Optical rotations: JASCO DIP-370 digital polarimeter. IR Spectra: Bio-Rad FTS-135 spectrometer, KBr pellets, in cm^{-1}. NMR spectra: Bruker AM-400 instrument (400/100 MHz), and Bruker DRG-500 instrument (500/125 MHz); δ in ppm rel. to TMS as internal standard, J in Hz. ESI-MS: Finnigan MAT 90 instrument; in *m/z*. HR-ESI-MS: API Qstar Pulsar LC/TOF instrument.

Plant material The roots of *S. tuberosa* used in this investigation were collected from Wenshan country, Yunnan Province, P. R. China, in December 2008. The plant species was authenticated by Prof. Gun Gong, and the voucher specimen (BN171) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The dried and powdered roots of *S. tuberosa* (20 kg) were extracted with MeOH. After evaporation of the collected percolate, the crude extract was acidified with dilute HCl (4%) to pH 1－2 and partitioned between $CHCl_3$ and water. The aqueous part was basified with aqueous NH3 to pH 9－10 and extracted with $CHCl_3$ to afford 80 g of crude alkaloids. Then, the crude alkaloids were applied to a silica gel CC eluting with gradient mixtures of petroleum ether-acetone (from 1∶0 to 0∶1) to give seven major fractions (Fr. **1**-Fr. **7**). Fr. **3** (30 g) was subjected to amino silica gel and silica gel CC, eluting with petroleum ether-acetone (from 10∶1 to 3∶1) to afford four major fractions, Fr. **3a**-Fr. **3d**. Fr. **3b** (2.7g) was further purified by silica gel CC and Sephadex LH-20 column to obtain **1** (19 mg). Fr. **4** (19 g) was applied to a MPLC, eluted with CH_3OH-H_2O to afford three major fractions, Fr. **4a**-Fr. **4c**. Fr. **4a** (2.2 g) was further purified by silica gel CC and Sephadex LH-20 column to obtain **2** (11 mg).

Dedrostemotinine (**1**), $C_{18}H_{23}NO_5$, $[\alpha]_D^{20}$ －7.6° (*c* 0.50, MeOH), colorless amorphous power. ^{13}C NMR data see Table 1. ^1H NMR data: ^1H NMR ($CDCl_3$, 500 MHz) δ_H 1.91 (1H, m, H-1a), 1.68 (1H, m, H-1b), 1.70 (1H, m, H-2a), 1.64 (1H, m, H-2b), 2.87 (1H, dd, J＝6.8, 6.4 Hz, H-3), 3.28 (1H, dd, J＝10.0, 15.0 Hz, H-5a), 3.03 (1H, dd, J＝15.1, 11.4 Hz, H-5b), 4.68 (1H, br s, H-6), 1.93 (1H, m, H-7a), 1.61 (1H, m, H-7b), 2.53 (1H, m, H-8a), 1.57 (1H, m, H-8b), 7.31 (1H, br s, H-10), 1.93 (3H, s, H-13), 4.28 (1H, ddd, J＝5.0, 7.0, 1.6 Hz, H-14), 2.37 (1H, m, H-15a), 1.47 (1H, m, H-15b), 2.66 (1H, dd, J＝3.0, 7.5 Hz, H-16), 1.26 (3H, d, J＝7.5 Hz, H-6).

Table 1 ^{13}C NMR data of compound **1** and **2** in $CDCl_3$ (^{13}C: 125 MHz; δ: ppm)

No.	1	2	No.	1	2
1	29.1 (t)	29.9 (t)	10	150.4 (d)	38.2 (t)
2	28.8 (t)	29.4 (t)	11	130.4 (s)	34.2 (d)
3	71.9 (d)	71.7 (d)	12	173.8 (s)	179.7 (s)
5	58.3 (t)	58.0 (t)	13	10.7 (q)	15.5 (q)
6	79.0 (d)	77.3 (d)	14	83.4 (s)	82.9 (s)
7	29.0 (t)	29.7 (t)	15	33.9 (t)	33.9 (t)
8	27.7 (t)	26.6 (t)	16	35.3 (d)	35.2 (d)
9	87.9 (s)	83.3 (s)	17	179.4 (s)	179.0 (s)
9a	106.3 (s)	106.6 (s)	18	14.8 (q)	14.9 (q)

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